

## **REMARKS**

### Amendment

Attached is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

### Specification

The title of the instant application has been changed to "Method of Inhibiting Angiogenesis By Administration of A Corticotropin Releasing Factor Receptor 2 Agonist".

Figure 1D was objected to because the letter "C" is not visible. Applicants hereby submit a new Figure 1D that has a visible letter "C". Applicants also submit herein amended Brief Description of the Drawings for Figures 4 and 6.

### The 35 U.S.C. §112 Rejection

Claims 20-23 were rejected under 35 U.S.C. §112, first paragraph, for lack of enablement. The rejection is respectfully traversed.

The present invention provides data that indicate Corticotropin Releasing Factor Receptor 2 (CRFR2) null mutant mice exhibit an increase in the size and number of blood vessels in various tissues. Figure 7 shows an increase in number and size of blood vessels in the anterior pituitary (Figure 7B), white adipose tissue (Figure 7D) and dorsal brain surface (Figure 7F) in CRFR2 null mutant mice. Microfil perfused tissues also indicate increased vessel size and number in dorsal brain surface (Figure 9A), large intestine (Figure 9B) and heart (Figure 9C). The major vessels in kidney, adrenal glands and testis are significantly increased in size in CRFR2 null mutant mice relative to those of control mice (Figure 10). Since CRFR2 receptor and its activity have been localized within the endothelial cell layer of blood vessels, Applicants' data indicates that CRFR2 plays a significant role in regulating angiogenesis. In view of the data disclosed herein, one of ordinary skill in the art would conclude that well-known CRFR2 agonists such as urocortin and CRF could be used to inhibit angiogenesis.

The Examiner argued that many anti-angiogenic therapies, particularly for treating cancer, were highly active in animal models, but clinical results so far have been disappointing

(Griffioen et al., *Pharmacological Review* 52:237-268, 2000).

Applicants respectfully disagree.

With regard to clinical trials investigating alteration of angiogenesis for treatment of cancer, clinical trials have been initiated to evaluate an anti-VEGF monoclonal antibody and a VEGFR-2 antagonist as therapy for patients with different types of solid tumors. As a result of favorable results from Phase 1 and 2 studies, randomized multicenter clinical investigations of angiogenesis inhibitors are ongoing (Rosen, *Cancer Journal* 7 Suppl 3:S120-8, 2001). Furthermore, the instant specification teaches alterations in angiogenesis could be a useful treatment for cardiovascular disease. Encouraging results have been obtained with VEGF and bFGF studies in animal models, leading to clinical trials in ischemic heart disease (Khurana et al., *Hypertension* 38:1210-16, 2001). Recent data has also shown success in the application of VEGF gene therapy strategies for therapeutic angiogenesis in critical limb ischemia and myocardial ischemia in clinical setting (Francis et al., *Physiological Genomics* 7:79-94, 2001).

The Examiner argued that in animal models of either tumor growth or inflammatory angiogenesis, the majority of the vasculature is in a proangiogenic state. In contrast, in human tumors

the percentage of proangiogenic vessels is variable, often quite low, and hence anti-angiogenic therapy may only affect a minority of vessels (Griffioen et al., p262). Applicants respectfully disagree.

Tumor angiogenesis is essential for the growth of primary and metastatic tumors. For tumors to create a neovascular blood supply, tumor cells need to secrete proangiogenic factors in order to overcome the inhibitory angiogenic factors. Therefore, targeting such inhibitors would allow for tumor growth suppression (Ellis et al., *Seminars in Oncology* 28:94-104). It is the balance between proangiogenic and antiangiogenic molecules in the microenvironment of a vessel *in vivo* that determine whether the existing vasculature will expand, remain the same, or regress (Jimenez et al., *Journal of Molecular Medicine* 78:663, 2001).

The Examiner also argued that strategies to target specific stages of the disease progression and hence angiogenesis require enormous preclinical research effort on the most potent formulations, dosing regimens, and so on, and therefore, clinical applications are not expected to start soon (Griffioen et al., p262). Applicants respectfully disagree.

Applicants submit that the cited reference is not referring to common methodologies of drug treatment such as IV

infusion that are well known in the art and do not require any "special skills" to practice. For treatment of cardiovascular disease, one of ordinary skill in the art would readily employ methods of local transfer since local transfer of an agent such as VEGF by perivascular or intravascular delivery provides a way of enhancing arterioprotective endothelial functions without stimulating neovascularization at other sites (Laitinen et al., *Human Gene Therapy* 8:1737, 1997).

Based on the data contained herein, Applicants respectfully submit that the claims on the method of inhibiting angiogenesis reasonably correlate to the scope of the enablement provided by the specification. Accordingly, Applicants respectfully request that the rejection of claims 20-23 under 35 U.S.C. §112, first paragraph, be withdrawn.

Claims 20-23 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite. The rejection is respectfully traversed.

Claim 20 has been amended to recite a step that relates back to the preamble. Claims 20 and 21 have also been amended to recite the full names for the acronyms "CRFR2" and "CRF".

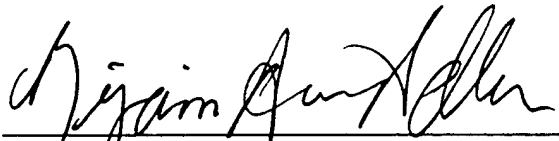
Accordingly, Applicants respectfully request that the rejections of claims 20-23 under 35 U.S.C. §112, second paragraph, be withdrawn.

This is intended to be a complete response to the Office Action mailed August 13, 2001. If any issues remain outstanding, the Examiner is respectfully requested to telephone the undersigned attorney of record for immediate resolution.

Respectfully submitted,

Date:

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Benjamin Aaron Adler, Ph.D., J.D.  
Registration No. 35,423  
Counsel for Applicant

ADLER & ASSOCIATES  
8011 Candle Lane  
Houston, Texas 77071  
(713) 270-5391  
badler1@houston.rr.com

VERSION WITH MARKINGS TO SHOW CHANGES MADE

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IN THE SPECIFICATION:

The title of the instant application on page 1 has been amended as follows:

~~CORTICOTROPIN-RELEASING FACTOR RECEPTOR 2 DEFICIENT MICE AND USES THEREOF~~ METHOD OF INHIBITING ANGIOGENESIS BY ADMINISTRATION OF A CORTICOTROPIN RELEASING FACTOR RECEPTOR 2 AGONIST

Lines 17-19 on page 11 has been amended as follows:

~~Figures 4A-4D demonstrate the~~ Figure 4 shows increased anxiety-like behavior of mutant animals in the elevated plus maze; and open field tests. (control n=7, mutant n=7; mean  $\pm$  SEM).

Line 20, page 11 to line 2, page 12 has been amended as follows:

**Figure 4A:** shows the percentage Percentage of time spent in the open arms (\*\*,  $p < 0.005$ ) and number of entries visits to the open arms (\*,  $p < 0.02$ ) were significantly less for the male mutant mice

than for the wild type controls (control n=7, mutant n=7; mean +SEM).

Lines 3-11 on page 12 has been amended as follows:

**Figure 4B:** ~~Locomotor activity was not different between control and mutant animals as measured by total closed arm entries ( $p=0.64$ ) and total arm entries ( $p=0.38$ ).~~ shows the percentage of time spent in the open arms (\*,  $p<0.03$ ) and number of entries to the open arms (\*,  $p<0.03$ ) were significantly less for the female mutant mice than for the wild type controls (control n=9, mutant n=12; mean +SEM).

**Figure 4C:** ~~No differences were found in anxiety-like behavior measured in the light/dark box experiment for time spent in light portion of the box.~~ shows locomotor activity in the EPM was not different between control and male mutant animals as measured by total closed arm entries and total arm entries.

**Figure 4D:** ~~No differences were found in anxiety-like behavior measured in the light/dark box experiment for the number of transitions between the light and dark portions.~~ shows locomotor activity in the EPM was not different between control and female

mutant animals as measured by total closed arm entries and total arm entries.

Figure 4E shows no difference was found in anxiety-like behavior as measured in the light/dark box experiment for time spent in the light portion of the box.

Figure 4F shows no difference was found in anxiety-like behavior as measured in the light/dark box experiment for the number of transitions between the light and dark portions.

Figure 4G shows increased anxiety-like behavior of mutant mice in the open field test as measured by the time spent in the inner squares of the open field apparatus (\*,  $p < 0.05$ , control  $n = 5$ , mutant  $n = 7$ , mean  $\pm$  SEM).

Figure 4H shows increased anxiety-like behavior of mutant mice in the open field test as measured by the percent of total crossings occurring in the inner squares of the open field apparatus (\*\*,  $p < 0.01$ , control  $n = 5$ , mutant  $n = 7$ , mean  $\pm$  SEM).

Lines 6-9 on page 13 has been amended as follows:

~~Figure 6 shows cardiovascular responses to intravenous infusion of 1.0  $\mu$ g urocortin in wild type ( $n = 5$ ) and mutant mice ( $n = 3$ ). Note the remarkable muted response of mutant mice to the~~

urocortin injection. \*\*\*  $p < 0.005$ . cardiovascular responses to intravenous infusion of 1.0 ug UCN in wild type (n=5) and mutant mice (n=3) (white bar). Note the remarkable muted response of mutant mice to the UCN injection. \*\*\*  $p < 0.005$ . *Crfr2* mutant mice also received a second infusion of sodium nitroprusside (0.8 ug in 100 ul of 0.9% saline) following recovery of arterial pressure from the UCN infusion (black bar). The mean arterial pressure (MAP) was determined from the blood pressure tracings.

#### **IN THE CLAIMS:**

Claim 20 has been amended as follows:

20. (amended) A method of inhibiting angiogenesis in a target tissue comprising the step of administering a Corticotropin Releasing Factor Receptor 2 (CRFR2) agonist to said target tissue, wherein said CRFR2 agonist inhibits angiogenesis in said tissue.

Claim 21 has been amended as follows:

21. (amended) The method of claim 20 wherein said CRFR2 agonist is selected from the group consisting of urocortin and corticotropin releasing factor CRF.